## ABSTRACT OF THE DISCLOSURE

Methods and compositions for producing single-stranded cDNA (ss-cDNA) with a vector-based system in eukaryotic cells. In one embodiment, the vector comprises plasmid(s) that contain a reverse transcriptase/RNAse H gene and a cassette, which includes a sequence coding for a sequence of interest and an inverted repeat, which produces an RNA template from which the reverse transcriptase synthesizes ss-cDNA of a specified sequence. The ss-cDNA forms a "stem-loop" structure as a result of the inverted tandem repeat, forming a double stranded DNA stem with the sequence of interest in the loop. The double-stranded stem may also contain one or more restriction endonuclease recognition sites cleaved by the desired corresponding restriction endonuclease(s) so that the loop portion, or sequence of interest, is released as single-stranded DNA. The plasmid also includes a second sequence of interest 3' to the inverted repeats which is likewise produced with minimal vector sequence. *In vivo* transfections show expression of reverse transcriptase(s)/RNase H(s) within eukaryotic cells as well as synthesis of RNA transcripts which formation of the ss-cDNA for such therapeutic purposes as gene inactivation using duplex or triplex binding of nucleic acids, site-directed mutagenesis, interruption of cellular function by binding to specific cellular proteins, and interfering with RNA splicing functions.